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Comprehensive biomarker profiling in patients with obstructive sleep apnea

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ABSTRACT

Objectives: The pathophysiological links between obstructive sleep apnea syndrome (OSAS) and cardiovascular mortality are incompletely understood. We aimed to contribute to a better characterization by using comprehensive biomarker profiling quantifying hemodynamic cardiac stress, cardiomyocyte injury, inflammation, endothelial function, matrix turnover and metabolism.

Design and methods: In 65 patients with moderate or severe OSAS [apnea–hypopnea index (AHI) 39 ± 20/h] and 33 patients with no or mild OSAS (AHI 8 + 4/h), B-type natriuretic peptide (BNP), N-terminal-pro-BNP (NT-proBNP), high-sensitivity cardiac troponin I (hs-cTnI), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and insulin were measured before and after sleep. In a subgroup measurements were repeated in a second night with continuous positive airway pressure (CPAP).

Results: Patients with moderate/severe OSAS had higher insulin before sleep [median (interquartile range), 36.4 (21.9–52.1) vs. 20.8 (10.6–32.8) mU/mL; p = 0.006], higher IL-6 after sleep [1.00 (0.73–1.58) vs. 0.72 (0.48–0.94) pg/mL; p = 0.005], and larger relative overnight reduction in BNP [−9 (−35–0) vs. −3 (−21–13)%; p = 0.04] than those with mild/no OSAS. Insulin before sleep was the only independent predictor of moderate/severe OSAS. Insulin before and IL-6 after sleep were independent predictors of severe OSAS, and when combined provided high diagnostic accuracy for severe OSAS (area under the receiver operator characteristic curve 0.80; 95%-confidence interval 0.69–0.91). In contrast, there were no significant differences in NT-proBNP, hs-cTnI, VEGF, and MMP-9 between moderate/severe and mild/no OSAS. Short-term CPAP had no impact on biomarker concentrations before and after sleep.

Conclusions: Significant OSAS is characterized by a distinct biomarker profile including high insulin before and high IL-6 after sleep.

Introduction

There is a large body of epidemiologic evidence linking the obstructive sleep apnea syndrome (OSAS) with important cardiovascular conditions including hypertension, metabolic syndrome, coronary artery disease, arrhythmia, and heart failure [1]. Given the potentially serious consequences of untreated severe OSAS [2], timely recognition, risk stratification, and appropriate treatment are crucial. In contrast to other cardiovascular conditions such as coronary artery disease [3] and heart failure [4], the pathophysiological links between OSAS and cardiovascular mortality as well as the potential role of biomarkers in the characterization and diagnosis of disease are incompletely understood [5]. Previous studies on the association between biomarkers and presence and severity of OSAS have revealed conflicting results. For example, some studies have reported higher B-type natriuretic peptide (BNP) or N-terminal-pro-B-type natriuretic peptide (NT-proBNP) in patients with OSAS than in those without [6], and a reduction in BNP/

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NT-proBNP following treatment with continuous positive airway pressure (CPAP) ventilation [7] or oral appliances [8], whereas others have found no such association [9–12]. One possible reason for such discrepant findings may be the existence of a relevant diurnal variation of biomarker plasma concentrations in OSAS, a phenomenon which has only rarely been studied [13,14]. In addition, a comprehensive multimarker profile including markers reflecting different aspects of pathophysiology may be more useful than the measurement of single markers. Therefore, we aimed to contribute to a better characterization and understanding of OSAS pathophysiology by using comprehensive biomarker profiling quantifying hemodynamic cardiac stress, cardiomyocyte injury, inflammation, endothelial function, matrix turnover and metabolism both before and after sleep and before and after CPAP therapy.

Methods

Patients and protocol

This was a prospectively conducted cross-sectional study performed at the University Hospital Basel, Switzerland, during an 18 month period. We measured biomarker concentrations in venous plasma before and after sleep in 98 consecutive patients referred to the sleep laboratory for evaluation of possible OSAS. Patients with a diagnosis of heart failure and/or symptoms or signs suggestive of heart failure were excluded. Such symptoms and signs included exertional dyspnea NYHA class II or more, nocturnal dyspnea, orthopnea, leg edema combined with pulmonary congestion, and distended neck veins. The study was approved by the institutional review board and carried out according to the principles of Good Clinical Practice. Written informed consent was obtained from all participating patients.

Clinical and laboratory assessment

Patient history was obtained with the help of a structured interview, and clinical assessment was performed including physical examination, and measurement of height, body weight, blood pressure, heart rate, and biomarkers. A venous blood sample from an antecubital vein was obtained in all patients between 8 p.m. and 10 p.m. (before sleep) and between 6 a.m. and 7 a.m. (after sleep) at night 1 (baseline). In a subgroup of patients, venous samples were obtained before (between 8 p.m. and 10 p.m.) and after (between 6 a.m. and 7 a.m.) sleep of a second night with nocturnal application of CPAP. Samples were collected in plastic tubes containing EDTA, placed on ice, and centrifuged at 3000 *g*; plasma was frozen at −80 °C. All plasma samples were analyzed by laboratory technicians who were blinded to patient characteristics and the results of the sleep studies. BNP and NT-proBNP concentrations were available for all patients. Concentrations of all other markers were available in 71 patients.

BNP plasma concentrations were determined using the commercially available AxSYM BNP assay (Abbott Laboratories, Zug, Switzerland) [15]. Plasma concentrations of NT-proBNP were determined with a quantitative electrochemiluminescence immunoassay (Elecsys proBNP, Roche Diagnostics GmbH, Basel, Switzerland) [16]. Plasma concentrations of insulin were determined with a quantitative electrochemiluminescence immunoassay (COBAS e601, Roche Diagnostics, Basel, Switzerland). Cardiac troponin I (cTnI) was measured using an established high-sensitivity immunoassay (Erenna® hs-cTnI Immunoassay, Singulex, Alameda, CA, USA) [17]. The assay has a lower limit of detection of 0.04 pg/mL, a lower limit of quantification of 0.24 pg/mL, and an inter-assay coefficient of variation (CV) of 7%. The following plasma biomarkers (lower limit of quantification, inter-assay CV) were additionally determined with the high sensitivity Erenna® immunoassay System: interleukin-6 (IL-6; 0.03 pg/mL, 15%), vascular endothelial growth factor (VEGF, 0.2 pg/mL, 4%), and matrix metalloproteinase-9 (MMP-9, 0.78 pg/mL, 15%).

Assessment of sleep-related breathing and CPAP therapy

Overnight polysomnography or polygraphy (ResMed, Mönchengladbach, Germany) including measurement of nasal flow, snoring, abdominal and thoracic movements, and oxygen saturation (SaO_2) was performed in all patients. A more than 50% reduction of airflow lasting for at least 10 s accompanied by a 4% decrease of SaO_2 (compared to a preceding stable SaO_2) was scored as hypopnea, and apnea was defined as the cessation of airflow for at least 10 s. The numbers of apneic and hypopneic episodes per hour of estimated sleep time were reported as apnea–hypopnea index (AHI). An obstructive apnea was defined as the absence of airflow in the presence of thoracic and abdominal movements, while flow limitation in the inspiratory flow signal or the occurrence of snoring was scored as obstructive hypopnea. The presence of OSAS was defined as an AHI > 5 events per hour. OSAS was graded according to AHI as mild (AHI < 15/h), moderate (AHI ≥ 15/h, <30/h), or severe (AHI ≥ 30/h). All polygraphic recordings were scored by a sleep technician who was unaware of the results of the echocardiographic studies.

Nocturnal CPAP ventilation was offered to selected patients with moderate or severe OSAS using the Autoset® CS system (ResMed, Mönchengladbach, Germany). The Autoset device measures mask airflow with a pneumotachograph. The flow is processed to determine respiratory airflow, leaks, and snore. The pressure is set at 4 cm H_2O at the beginning of the study. The pressure increases according to algorithms in response to apneas, snoring, and episodes of flow limitation suggested by the shape of the inspiratory flow wave. The maximum pressure limit is 20 cm H_2O. In the absence of respiratory disordered breathing, the pressure is allowed to decrease slowly to 4 cm H_2O.

Statistical analysis

Data are presented as frequencies and percentages, mean ± standard deviation, or median (interquartile range). Clinical characteristics and sleep study results were compared between patients with moderate/severe OSAS and those with mild/no OSAS using χ^2 tests, unpaired *t*-test, or Mann–Whitney-U test, as appropriate. Biomarker concentrations are given as median (interquartile range) and were compared between moderate or severe OSAS and mild or no OSAS using unpaired *t*-tests if plasma concentrations had a normal distribution and using Mann–Whitney-U tests if they had a skewed distribution. Kolmogorov–Smirnov-tests were used to assess whether there was a normal distribution. The following biomarkers had a normal distribution: percentage overnight change (Δ%) in BNP (Δ%BNP), Δ%NT-proBNP, Δ%hs-cTnI, VEGF before sleep, absolute overnight change (Δ) in VEGF (ΔVEGF), IL-6 before sleep, ΔIL-6, MMP-9 before sleep, and ΔMMP-9. All other biomarkers had a skewed distribution. All biomarker concentrations measured in the second night were compared between patients with moderate/severe OSAS and those with mild/no OSAS using Mann–Whitney-U tests because of the small sample size. Biomarker concentrations before and after sleep and in the first and second nights within the same subjects were compared using Wilcoxon-rank-tests. Spearman correlation coefficients were calculated for correlations of interest. Multivariable logistic regression including biomarker concentration (after ln-transformation if appropriate) as covariates was performed to identify predictors of moderate/severe and severe OSAS. Areas under the receiver operator characteristic curve (AUC) for biomarkers for the prediction of moderate/severe and severe OSAS were constructed. Analyses were performed using a commercially available statistical package (SPSS 19.0., Inc., Chicago, Illinois, USA).

Results

Clinical and sleep characteristics of the study population

Among the 98 patients included in the study, 65 had moderate/severe OSAS (AHI 39 ± 20/h), and 33 had mild/no OSAS (AHI 8 ± 4/h;

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$p < 0.001$). Clinical characteristics and sleep study findings in patients with moderate/severe OSAS versus those with mild/no OSAS are presented in Table 1. Patients with moderate/severe OSAS were more likely to be male and to take beta-blockers, and had higher body mass index than those with mild/no OSAS. As expected desaturation index and time spent with an arterial oxygen saturation below 90% (t90) were higher in patients with moderate/severe OSAS than in those with mild/no OSAS. Blood pressure and heart rate before and after sleep and changes overnight did not differ between the groups.

Natriuretic peptides

Before sleep, BNP plasma concentrations did not differ between patients with moderate/severe OSAS and those with mild/no OSAS (Table 2). There was a significant overnight decrease in BNP in patients with moderate/severe OSAS ($p < 0.001$; Table 2) but not in those with mild/no OSAS ($p = 0.27$; Table 2) with larger overnight relative reduction in BNP in those with moderate/severe OSAS ($p = 0.04$; Table 2). The absolute overnight changes in BNP did not differ between the groups. Concentrations of NT-proBNP before sleep did not differ between patients with moderate/severe and mild/no OSAS. In both groups, there was a significant decrease in NT-proBNP overnight ($p < 0.001$ and $p = 0.004$ respectively), and changes in NT-proBNP concentrations during sleep and concentrations after sleep did not differ between groups (Table 2).

Table 1
Baseline characteristics of patients with moderate or severe obstructive sleep apnea syndrome (OSAS) and those with none or mild OSAS.

	Moderate/severe OSAS (AHI ≥ 15/h) N = 65	None/mild OSAS (AHI < 15/h) N = 33	p value
Age (years)	54 ± 12	50 ± 17	0.15
Male gender	53 (82%)	18 (55%)	0.005
Body mass index (kg/m^2)	31.8 ± 6.3	28.0 ± 6.5	0.007
Serum creatinine (μmol/L)	79 ± 13 (n = 31)	73 ± 14 (n = 17)	0.17
Creatinine clearance (mL/min)	131 ± 49	119 ± 35	0.47
Co-morbidities			
Coronary artery disease	11 (17%)	2 (6%)	0.14
Left ventricular ejection fraction (%)	60 ± 5	58 ± 8	0.69
Diabetes mellitus	6 (9%)	3 (9%)	0.98
Chronic obstructive lung disease	10 (15%)	4 (12%)	0.70
Smoking	21 (32%)	12 (36%)	0.65
Medication			
ACEI/ARB	22 (34%)	7 (21%)	0.20
Beta-blockers	17 (26%)	3 (9%)	0.048
Diuretic	16 (25%)	3 (9%)	0.07
Calcium channel blocker	8 (12%)	2 (6%)	0.33
Aspirin	12 (18%)	3 (9%)	0.22
Oral anticoagulation	4 (6%)	3 (9%)	0.59
Statin	18 (28%)	7 (21%)	0.54
Inhaled steroids	6 (9%)	2 (6%)	0.62
Systemic steroids	2 (3%)	0	0.32
Beta-2-mimetics	6 (9%)	2 (6%)	0.62
Epworth sleepiness scale	11 ± 5	12 ± 4	0.64
Sleep time (min)	364 ± 75	339 ± 73	0.13
AHI (events/h)	39 ± 20	8 ± 4	<0.001
Time with SaO_2 <90% (min)	25.6 (7.8–100.1)	1.9 (0–22.5)	0.001
Desaturation index	30.0 (15.4–50.0)	6.7 (3.2–11.5)	<0.001
Systolic blood pressure (mm Hg)			
Before sleep	140 ± 18	139 ± 19	0.68
After sleep	133 ± 19	128 ± 20	0.29
Change overnight	−8 ± 17	−10 ± 12	0.54
Diastolic blood pressure (mm Hg)			
Before sleep	81 ± 11	78 ± 9	0.15
After sleep	77 ± 13	74 ± 11	0.18
Change overnight	−4 ± 12	−4 ± 8	0.91
Heart rate (bpm)			
Before sleep	73 ± 11	75 ± 11	0.46
After sleep	65 ± 9	63 ± 9	0.58
Change overnight	−7 ± 14	−12 ± 10	0.11

Data are given as numbers and percentages, mean ± standard deviation, or median (interquartile range) as appropriate.
Abbreviations: AHI: apnea hypopnea index, ACE/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker, SaO_2: arterial oxygen saturation.

High sensitivity cardiac troponin

Plasma concentrations of hs-cTnI before and after sleep did not differ between patients with moderate/severe and mild/no OSAS (Table 2). There were no significant changes in hs-cTnI overnight in either group (moderate/severe OSAS: $p = 0.73$, mild/no OSAS: $p = 0.36$).

Interleukin-6

Before sleep, IL-6 plasma concentrations did not significantly differ between patients with moderate/severe OSAS those with mild/no OSAS although there was a trend toward higher IL-6 in patients with moderate/severe OSAS ($p = 0.08$; Table 2). There was a significant reduction in IL-6 plasma concentrations overnight in both patients with moderate/severe ($p = 0.01$) and mild/no OSAS ($p = 0.001$). Changes in IL-6 overnight did not significantly differ between groups. After sleep, IL-6 was significantly higher in patients with moderate/severe OSAS than in those with mild/no OSAS ($p = 0.005$). If patients with severe, moderate, and mild/no OSAS were compared, there was an obvious dose–effect relationship between IL-6 concentrations and OSAS severity before and after sleep but this was stronger after sleep [before sleep: median (interquartile range), 1.41 (0.74–2.16) vs. 1.22 (0.78.1.69) vs. 1.00 (0.63–1.38) pg/mL; $p = 0.08$, after sleep: 1.22 (0.82–1.81) vs. 0.83 (0.70–1.06) vs. 0.72 (0.48–0.94) pg/mL; $p = 0.03$]. There were significant correlations between higher IL-6 measured before sleep and higher desaturation index ($r = 0.28$; $p = 0.02$) and higher t90 ($r = 0.33$; $p = 0.006$). There were even stronger correlations between higher IL-6 after sleep and higher desaturation index ($r = 0.44$; $p < 0.001$) and higher t90 ($r = 0.45$; $p < 0.001$), and there was also a significant correlation between higher IL-6 after sleep and higher AHI ($r = 0.38$; $p = 0.001$).

MMP-9

Before sleep, MMP-9 did not significantly differ between patients with moderate/severe and mild/no OSAS (Table 2). In neither group was there a statistically significant change in MMP-9 concentrations overnight (moderate/severe OSAS: $p = 0.6$, mild/none: $p = 0.08$). After sleep there was however a trend toward higher MMP-9 plasma concentrations in patients with moderate/severe OSAS compared those with mild/no OSAS, which just failed to reach statistical significance ($p = 0.06$, Table 2).

VEGF

Before sleep, VEGF plasma concentrations were similar in patients with moderate/severe and mild/no OSAS (Table 2). There was a significant decrease in VEGF overnight in both patients with moderate/severe ($p < 0.001$) and those with mild/no OSAS ($p = 0.003$). Changes in VGEF concentrations overnight and VEGF concentrations after sleep did not differ between the groups.

Insulin

Before sleep, insulin plasma concentrations were higher in patients with moderate/severe OSAS compared to those with mild/no OSAS (Table 2). There was a significant decrease in insulin overnight in both groups (moderate/severe OSAS: $p < 0.001$, mild/no OSAS: $p = 0.001$). However, the absolute overnight decrease in insulin was larger in

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Table 2
Biomarker concentrations in patients with moderate or severe obstructive sleep apnea syndrome (OSAS) and those with none or mild OSAS.

	Moderate/severe OSAS (AHI > 30/h) N = 65	Mild/no OSAS (AHI < 30/h) N = 33	p value for the comparison moderate/severe vs. mild/no OSAS
BNP			
Before sleep (pg/mL)	28 (16–54)	37 (20–58)	0.41
After sleep (pg/mL)	18 (14–49)*	29 (20–48)	0.07
Δ (pg/mL)	−3 (−14–0)	−1 (−10–3)	0.19
Δ% (%)	−9 (−35–0)	−3 (−21–13)	0.04
NT-proBNP			
Before sleep (pg/mL)	40 (16–96)	36 (21–69)	0.61
After sleep (pg/mL)	29 (17–82)*	31 (19–59)*	0.64
Δ (pg/mL)	−6 (−13–1)	−6 (−13–2)	0.74
Δ% (%)	−14 (−25–8)	−12 (−22–9)	0.92
Hs-cTnI			
Before sleep (pg/mL)	1.51 (1.03–2.37)	1.32 (0.82–2.78)	0.43
After sleep (pg/mL)	1.61 (1.10–2.43)	1.43 (0.73–3.03)	0.38
Δ (pg/mL)	0 (−0.33–0.18)	0.07 (−0.19–0.28)	0.29
Δ% (%)	0 (−15–12)	6 (−7–18)	0.49
VEGF			
Before sleep (pg/mL)	178 (95–243)	177 (76–342)	0.21
After sleep (pg/mL)	61 (41–152)*	87 (48–148)*	0.25
Δ (pg/mL)	−63 (−139 to −9)	−73 (−271 to −6)	0.16
Δ% (%)	−50 (−67 to −16)	−36 (−76 to −8)	0.73
IL-6			
Before sleep (pg/mL)	1.37 (0.78–1.99)	1.00 (0.63–1.38)	0.08
After sleep (pg/mL)	1.00 (0.73–1.58)*	0.72 (0.48–0.94)*	0.005
Δ (pg/mL)	−0.21 (−0.47–0.07)	−0.21 (−0.36–0.05)	0.84
Δ% (%)	−13 (−34–8)	−20 (−34 to −9)	0.21
MMP-9			
Before sleep (pg/mL)	30,261 (17,859–48,651)	23,157 (15,145–44,364)	0.19
After sleep (pg/mL)	27,936 (18,523–42,670)	22,349 (12,941–29,117)	0.06
Δ (pg/mL)	−1452 (−14,179–9432)	−3653 (−15,839–1958)	0.66
Δ% (%)	6 (−41–45)	−23 (−45–18)	0.18
Insulin			
Before sleep (mU/mL)	36.4 (21.9–52.1)	20.8 (10.6–32.8)	0.006
After sleep (mU/mL)	13.3 (8.2–18.9)*	10.2 (5.7–14.5)*	0.11
Δ (mU/mL)	−21.4 (−40.4 to −7.0)	−6.3 (−18.7 to −3.3)	0.009
Δ% (%)	−61 (−76 to −41)	−43 (−68 to −31)	0.07

Data are given as median (interquartile range).
BNP: B-type natriuretic peptide, NT-proBNP: N-terminal-pro-B-type natriuretic peptide, IL-6: interleukin-6, VEGF: vascular endothelial growth factor, MMP-9: matrix metalloproteinase-9, cTnI: cardiac troponin I.
Δ: overnight change in absolute terms, Δ%: relative overnight change in %.
* $p < 0.05$ for the change of biomarker concentration overnight.

moderate/severe OSAS compared to mild/no OSAS, and morning insulin concentrations did not significantly differ between groups anymore.

Acute effects of CPAP

In 21 patients, BNP and NT-proBNP plasma concentrations were measured in the second night before and after sleep during which CPAP was applied, and in 12 patients, IL-6, VEGF, MMP-9, cTnI, insulin were measured in the second night before and after sleep during which CPAP was applied. Plasma concentrations of all markers before and after sleep and changes during sleep did not significantly differ between the nights with and without CPAP (Table 3).

Biomarkers for the prediction of significant OSAS

Male gender, higher body mass index, higher insulin before sleep, higher IL-6 after sleep, and smaller Δ%BNP (more pronounced decrease) were significant predictors of moderate/severe OSAS in the univariable logistic regression model (Table 4). In the multivariable model, higher insulin before sleep was the only independent predictor of moderate/severe OSAS (Table 4). The IL-6 concentration after sleep just failed to remain in the model ($p = 0.05$). The AUC for insulin before sleep to predict moderate/severe OSAS was 0.74 (95% CI, 0.62–0.87). The optimal cut-off of insulin before sleep of 22.36 mU/mL had a sensitivity of 76% and a specificity of 65% for the prediction of moderate/severe OSAS.

If severe OSAS rather than moderate/severe OSAS was used as the dependent variable, male gender, hypertension, beta-blocker use, diuretic use, higher body mass index, higher insulin before sleep and higher IL-6 before and after sleep were significant predictors in the univariable analysis (Table 5). In the multivariable model, only higher insulin before sleep and higher IL-6 after sleep were independently associated with severe OSAS (Table 5). The AUCs for insulin before sleep and IL-6 after sleep were 0.77 (95% CI, 0.66–0.88) and 0.71 (95% CI, 0.59–0.84) respectively. The best cut-off for insulin before sleep of 24.29 mU/mL had a sensitivity of 85% and a specificity of 61%. The best cut-off for IL-6 after sleep of 0.828 pg/mL had a sensitivity of 77% and a specificity of 59%. A continuous combination variable constructed from insulin before sleep and IL-6 after sleep readings had an AUC of 0.80 (95% CI, 0.69–0.91; Fig. 1). A score built based on these two markers (0: both marker below cut-off, 1: only insulin before sleep above cut-off, 2: only IL-6 after sleep above cut-off, 3: both markers above cut-off) had an AUC for the prediction of severe OSAS of 0.81 (95% CI, 0.70–0.92).

Discussion

In the present study, we have shown that significant OSAS is associated with a distinctive biomarker profile characterized by higher insulin before sleep, higher IL-6 after sleep, and a more pronounced overnight reduction in BNP than in those without. In contrast, we could find no significant differences in NT-proBNP, cTnT, MMP-9, and VEGF between patients with moderate/severe OSAS and those with mild/no OSAS,

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Table 3
Effects of short-term continuous positive airway pressure (CPAP) on biomarkers before and after sleep and changes overnight.

	No CPAP	CPAP	p value
BNP (pg/mL; n = 21)			
Before sleep	22 (14–42)	23 (14–33)	0.23
After sleep	14 (14–24)	14 (14–29)	0.80
Δ	−5 (−13–0)	−2 (−9–0)	0.29
NT-proBNP (pg/mL; n = 21)			
Before sleep	40 (20–89)	49 (24–71)	0.18
After sleep	29 (21–70)	31 (19–58)	0.07
Δ	−8 (−19–1)	−12 (−19–1)	0.79
cTnI (pg/mL; n = 12)			
Before sleep	2.26 (1.67–2.82)	2.19 (1.42–2.93)	0.53
After sleep	1.78 (1.41–3.37)	2.09 (1.19–3.03)	0.27
Δ	−0.04 (−0.40–0.57)	0.04 (−0.27–0.39)	0.43
VEGF (pg/mL; n = 12)			
Before sleep	196 (96–307)	185 (90–280)	0.59
After sleep	61 (51–189)	73 (59–140)	0.86
Δ	−120 (−246 to −40)	−122 (−191 to −24)	0.93
IL-6 (pg/mL; n = 12)			
Before sleep	1.32 (0.82–2.03)	1.40 (0.89–1.85)	0.81
After sleep	1.06 (0.81–1.54)	1.15 (0.83–1.51)	0.58
Δ	−0.16 (−0.47–0.07)	−0.13 (−0.61–0.01)	0.10
MMP-9 (pg/mL; n = 12)			
Before sleep	33,317 (15,375–56,990)	34,007 (18,488–70,556)	0.48
After sleep	25,025 (21,212–39,551)	24,021 (7571–50,430)	0.88
Δ	−15,608 (−30,064–16,703)	−10,380 (−23,721 to −5672)	0.31
Insulin (mU/mL; n = 12)			
Before sleep	52.1 (35.9–84.7)	59.2 (17.9 to −128.9)	0.48
After sleep	18.9 (14.6–25.0)	15.5 (13.4–27.2)	0.66
Δ	−35.9 (−68.0 to −20.8)	−38.2 (−55.7 to −6.2)	0.86

Data are given as median (interquartile range).
BNP: B-type natriuretic peptide, NT-proBNP: N-terminal-pro-B-type natriuretic peptide, IL-6: interleukin-6, VEGF: vascular endothelial growth factor, MMP-9: matrix metalloproteinase-9, cTnI: cardiac troponin I.
Δ: overnight change in absolute terms.

and short-term CPAP had no impact on biomarker concentrations. The combination of two biomarkers (insulin and IL-6) – one measured before sleep and the other measured after sleep – was associated with a moderate to high accuracy for the detection of severe OSAS, indicating that a multimarker approach also taking into account the diurnal variability of these markers may be a promising strategy to characterize patients with significant OSAS. Of course, our results need to be validated in an independent cohort before clinical application of our findings should be considered.

Our findings of higher IL-6 plasma concentrations in patients with moderate/severe OSAS compared to mild/no OSAS corroborate and extend that of a previous study [18]. To the best of our knowledge, this is the first study to assess diurnal variation of this parameter. Similarly, the association between OSAS and insulin resistance is well established [19], and the association between OSAS severity and insulin is not surprising. We were able to show however that the difference in IL-6 between patients with moderate/severe and mild/no OSAS is larger in the morning than in the evening. It may be speculated that this finding occurred as a result of higher oxidative stress during sleep in those with significant OSAS. On the other hand, a significant difference in insulin between patients with moderate/severe and mild/no OSAS was found only before sleep, possibly because during sleep there are fewer stimuli for insulin secretion. The combination of these markers is an example how we can make use of the diurnal variation of these markers. Of note, insulin remained an independent predictor of significant OSAS even if body mass index was put into the model.

In accordance with many [9–12] but not all studies we found no differences in BNP and NT-proBNP concentrations between patients with moderate/severe and mild/no OSAS. We had shown previously that appropriate CPAP therapy over several months had no impact on NT-proBNP either [20]. We observed a larger relative reduction in BNP during sleep in patients with moderate/severe compared to those with mild OSAS; no significant difference was found for the absolute changes however. No such effect was seen for NT-proBNP, which might have been due to the longer half-life of NT-proBNP compared to BNP. Given the absolutely very small difference between changes in BNP between patients with and without significant OSAS on one hand, and the high intra-individual biological variability of plasma BNP [21] on the other hand, this study must probably be interpreted in the way that BNP and NT-proBNP seem not to be useful markers to shed light on the mechanisms of increased cardiovascular risk in patients with OSAS.

Table 4
Predictors of moderate/severe obstructive sleep apnea syndrome (OSAS).

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Male gender	3.70 (1.45–9.31)	0.006	–	–
Body mass index	1.11 (1.03–1.21) per kg/m^2	0.01	–	–
Insulin before sleep	2.60 (1.23–5.48) per ln unit	0.01	2.60 (1.23–5.48) per ln unit	0.01
IL-6 after sleep	3.45 (1.25–9.53) per ln unit	0.02	–	–
Δ%BNP	0.98 (0.97–0.99) per %	0.04	–	–

OR: odds ratio, 95% CI: 95% confidence interval, IL-6: interleukin-6, Δ%BNP: relative overnight change in B-type natriuretic peptide.

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Table 5
Predictors of severe obstructive sleep apnea syndrome (OSAS).

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Male gender	3.04 (1.10–8.43)	0.03	–	–
Hypertension	2.64 (1.13–6.20)	0.03	–	–
Body mass index	1.16 (1.07–1.26) per kg/m^2	<0.001	–	–
Beta-blocker use	3.71 (1.32–10.43)	0.01	–	–
Diuretic use	4.42 (1.51–12.94)	0.007	–	–
Insulin before sleep	3.57 (1.66–7.65) per ln unit	0.001	4.04 (1.69–9.67) per ln unit	0.002
IL-6 after sleep	3.73 (1.50–9.29) per ln unit	0.005	3.82 (1.34–10.92) per ln unit	0.012
Δ%BNP	0.99 (0.97–1.01) per %	0.06	–	–

OR: odds ratio, 95% CI: 95% confidence interval, IL-6: interleukin-6, Δ%BNP: relative overnight change in B-type natriuretic peptide.

Although we used a high-sensitivity assay we found no differences in cTnI between patients with moderate/severe and mild/no OSAS. In a previous study, patients with more severe OSAS were found to have higher cTn, but this difference disappeared after adjustment for presence and severity of coronary artery disease [22]. Thus, although there is a large body of evidence suggesting an association between significant OSAS and cardiac stress, there seems to be no relevant myocardial injury as measurable by cTnI. Similarly, there were no differences in VEGF and MMP-9 concentrations between patients with moderate/severe and mild/no OSAS.

This study is limited by the fact that the sample size was small to moderate. However, we measured a comprehensive set of novel biomarkers before and after sleep, and in some subjects measurements were performed also before and after a night with CPAP application. Another limitation is related to the fact that we did not include a control group of healthy subjects but compared the severe/moderate and the no/mild OSAS groups. However, when comparing the severe/moderate OSAS group to a group of healthy controls, differences in clinical characteristics and biomarkers might have even been more significant although this remains speculative. Furthermore, the selection of the biomarkers in this study was arbitrary and may not have been perfect. Other markers such as high-sensitivity C-reactive protein or measures of nitric oxide might have been informative too in the present setting. In addition, we were unable to demonstrate an acute effect of CPAP on biomarkers. This does not exclude the possibility that biomarkers are affected by long-term CPAP use and might be useful to monitor the effect of therapy. We also acknowledge that the CPAP subgroup was relatively small. Further studies are required to find out which clinical consequences can be derived from the findings of the present study.

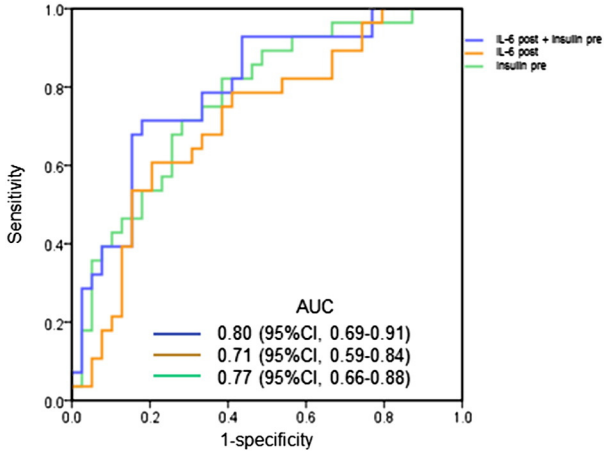

Fig. 1. Areas under the receiver operator characteristic curve [AUC; with 95% confidence intervals (95% CI)] for biomarker concentrations for the prediction of severe obstructive sleep apnea: IL-6 post: interleukin-6 plasma concentrations after sleep, insulin pre: insulin plasma concentrations before sleep, IL-6 post + insulin pre: continuous combination variable.

Conclusions

Significant OSAS is characterized by a distinct biomarker profile including high insulin before and high IL-6 after sleep. This profile could be helpful as a diagnostic tool, and possibly also contribute to novel therapeutic concepts. These findings also indicate that a pattern of biomarkers taking into account the diurnal variation of markers rather than the measurement of one single parameter might be appropriate to characterize the patient with significant OSAS.

Competing interests

JT and JE are employees of Singulex, the manufacturer of some of the biomarkers used in this study. CM has received financial support for his research from Singulex, the manufacturer of some of the biomarkers used in this study. The other authors have no competing interests.

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